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TITLE: Genome Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy

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14. ABSTRACT

The hypothesis that forms the basis for this research is that patients who possess certain SNPs or CNPs are at a greater risk for developing severe urinary morbidity or ED resulting from radiotherapy for prostate cancer. The specific aim of this project is to identify through a genome wide association study the SNPs and CNPs associated with the development of severe urinary morbidity and ED resulting from the use of radiation to treat prostate cancer. It should be noted that we may also identify SNPs or CNPs that are associated with protection against the development of these forms of radiation injury. Through the end of the project at the NYUMC site, substantial progress was made on the validation phase of the project. This included selection of approximately 5,000 SNPs and CNPs from the discovery phase of the GWAS for genotyping of the 600 samples that comprise the validation cohort. The genotyping and analysis are now being completed through a no-cost extension of the project at the Mount Sinai site.

15. SUBJECT TERMS
Radiation, SNP and CNP genotyping, normal tissue toxicities

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INTRODUCTION:

Radiotherapy can provide a sustainable cure for prostate cancer and has become accepted as a standard treatment option. However, some men develop side effects following treatment, including urinary morbidity, proctitis and erectile dysfunction, which have a substantial effect on quality of life. These side effects vary in duration and severity, and while most patients return to baseline symptom levels after a year, a subset of patients experience more severe and lasting effects. A predictive assay that could identify such patients could be used to help tailor treatment plans. Previous research on radiation induced injury in breast cancer patients suggests that the variation in such side effects is largely due to patient-specific, possibly genetic effects rather than treatment differences or random effects. The purpose of the current study is to identify genetic polymorphisms associated with development of urinary morbidity proctitis, or erectile dysfunction following radiotherapy for prostate cancer. The medical application of these findings will be to develop a risk assessment genetic test to assist physicians and patients in making informed decisions on the course of therapy for prostate cancer. Physicians and patients could together weigh the benefits of therapy with the individualized risk of developing radiation side effects and could then customize the treatment course.

BODY:

Year 1: Since a critical aspect of any association study is to insure that the cases and controls rigorously conform to the criteria for their selection, the main effort during the first year of the project was an intensive review of the clinical data for each subject in this study to verify their inclusion in this study. Thus, efforts were focused on the following tasks: patient follow-up, finalization of inclusion criteria, case definitions and preparation of high quality genomic DNA for microarray analysis. We completed patient follow-up pertaining to urinary outcomes (International Prostate Symptom Score, IPSS) and erectile dysfunction (Sexual Health Inventory for Men score and Mount Sinai Erectile Function Score) for the minimum time period for all individuals in our database. Case and control definitions were modified based on clinical characteristics of our patient set and findings in recently published reports.

Our database now includes over 3,000 men treated with brachytherapy and followed-up for a minimum of one year. We identified two replicate sets of 100 cases and 100 controls for each of the two outcomes required for genotyping analysis using the Affymetrix 6.0 SNP arrays. All patients were followed with assessment of urinary outcomes using the IPSS questionnaire and erectile dysfunction using the SHIM and MSEF questionnaires as planned. We collected blood samples and prepared genomic DNA from 726 patients. Demographic and clinical data for the 858 patients for whom we have DNA samples were analyzed to confirm that cases and controls were similar with respect to potential confounders (Table 1).

The patients included in the study were selected based on the criteria and case definitions outlined in the initial proposal with minor modifications based on clinical characteristics of the patients in our database and recently published findings regarding radiation injury outcomes. First, we decreased the minimum follow-up time for inclusion in the study from two years to one year. Our data as well as recent reports tracking the same radiotherapy adverse effects suggested that on average IPS scores and sexual function return to pre-treatment levels by 12 months post-treatment (Keyes, 2009; Aaltomaa, 2009; Tanaka, 2009). Twelve months appeared to be sufficient time to separate out those individuals who experience long-term symptoms that may have a genetic basis.

We also increased the number of patients included in the study. We initially planned to use a single set of 100 controls for both outcomes. After closer examination of the clinical characteristics of the patients, we found that it is appropriate to select a separate set of control patients for each outcome. We found that among the patients in our database, many exhibited one form of radiation injury and not the other suggesting that different genetic variants may contribute to the different outcomes, indicating that it is appropriate to study each outcome with a separate set of cases and controls.

Table 1. Demographic and clinical characteristics of cases and controls for urinary morbidity and erectile dysfunction controls.

		Urinary Morbidity	Erectile Dysfunction	
		N = 751	Cases N = 267	Controls N = 188
Age*, mean (sd)		64.4 (7.7)	63.2 (6.2)	60.6 (6.5)
Race, N(%)				
	Hispanic	55 (7.3%)	27 (10.1%)	10 (5.3%)
	Caucasian	570 (75.9%)	201 (75.3%)	150 (79.8%)
	African American	95 (12.6%)	28 (10.5%)	19 (10.1%)
	Asian	13 (1.7%)	6 (2.2%)	2 (1.1%)
	Not known	18 (2.4%)	5 (1.9%)	7 (3.7%)
Initial PSA, mean (sd)		8.1 (7.8)	10.3 (20.6)	7.1 (5.2)
Stage, N (%)				
	T1a	2 (0.3%)	1 (0.4%)	0
	T1b	2 (0.3%)	1 (0.4%)	0
	T1c	379 (50.5%)	118 (44.2%)	125 (66.5%)
	T2a	148 (19.7%)	51 (19.1%)	30 (15.9%)
	T2b	145 (19.3%)	61 (22.8%)	19 (10.1%)
	T2c	47 (6.3%)	24 (8.9%)	9 (4.8%)
	T3a	24 (3.2%)	10 (3.7%)	5 (2.7%)
	T3b	1 (0.1%)	0	0
	T3c	3 (0.4%)	1 (0.4%)	0
Gleason Score, N(%)	**3**	1 (0.1%)	1 (0.4%)	0
	4	7 (0.9%)	1 (0.4%)	2 (1.1%)
	5	27 (3.6%)	5 (1.9%)	4 (2.1%)
	6	423 (56.3%)	138 (51.7%)	127 (67.6%)
	7	205 (27.3%)	77 (28.8%)	45 (23.9%)
	8	64 (8.5%)	32 (11.9%)	8 (4.3%)
	9	20 (2.7%)	11 (4.1%)	2 (1.1%)
	10	4 (0.3%)	2 (0.7%)	0
Treatment Type				
	Implant Only	417 (55.5%)	121 (45.3%)	127 (67.5%)
	Implant + EBRT	324 (43.1%)	140 (52.4%)	60 (31.9%)
	EBRT Only	10 (1.3%)	6 (2.2%)	1 (0.5%)
Follow-up days, mean (min.,max.)		1707 (379, 4915)	1973 (379, 5482)	1658 (370, 4013)
Taking PDIs		-	113 (56.5%)	95 (53.1%)
Pre-treatment IPSS, mean (sd)		7.6 (6.0)	6.9 (5.4)	6.9 (5.7)
Pre-treatment SHIM, mean (sd)		-	20.0 (5.4)	22.3 (3.7)
Pre-treatment MSEF				
	0	-	7 (3.5%)	7 (3.9%)
	1	-	5 (2.5%)	0
	2	-	32 (16.1%)	25 (14.0%)
	3	-	155 (77.9%)	147 (82.1%)

We removed the constraint on ethnicity for inclusion in the study as requested by the DOD Human Research Protection Office (HRPO). We had initially restricted inclusion

to white, non-Hispanic patients in an effort to reduce identification of false positive associations due to population stratification. We identified several analytic methods and software programs, including principle components analysis and STRUCTURE and ADMIXMAP respectively, that are designed to determine ancestry based on genetic markers and to cluster individuals by genetic ancestry. Using this methodology we can assign a value for a genetic ancestry variable to each individual and control for population stratification in the tests for association.

We included patients in the study who were treated with either I-125 seed implant alone or in combination with external beam radiation therapy. There is no constant evidence in the literature to suggest that the effects on urinary or erectile function are different in the monotherapy versus the combination therapy (Lee, 2006; Hurwitz, 2008). Dosimetric measurements were collected for each patient and only patients whose dose to the prostate (D90) was within the range of 160-180 Gy were included regardless of treatment type.

We had initially planned to analyze urinary morbidity as a case-control outcome, but analysis of the distribution of IPSS scores showed that it would be more appropriate to treat this outcome as a quantitative trait. Thus, we treated the change in IPSS relative to pre-treatment as a continuous outcome measure of radiation-induced urinary morbidity, adjusting for pre-treatment score in genetic association tests. This definition allows for inclusion of individuals who report a less severe long-term response but, relative to their pre-treatment status, still experience a substantial decline in urinary symptoms. It also includes those patients who already had urinary problems prior to treatment but who still developed significant additional symptoms following treatment. This case definition better accounts for the subjective nature of the IPSS test, the normal distribution of IPS scores, and the variability in long-term urinary morbidity from moderate to severe.

With regard to erectile dysfunction, we had initially planned to exclude from the study patients who have taken phosphodiesterase inhibitors (PDIs) to treat erectile dysfunction as that may itself be associated causally with the outcome. Upon closer examination of the data we found that a substantial percentage of patients reported using PDIs, and if we included patients who reported using PDIs, there was only a small difference in usage between cases and controls. Rather than exclude these patients and reduce our sample size, we included these patients and control for PDI usage in the test for association.

As we updated our patient database and finalized our selection for inclusion in the GWAS, we found that we had sufficient numbers of proctitis cases (84 total) to also investigate this third form of radiation toxicity. The cases were identified as patients with proctitis grade of 2 or 3 as assessed by the Radiation Therapy Oncology Group (RTOG) toxicity grading scale. Controls were the remaining patients already included in the study who had RTOG proctitis grade 0 or 1. Thus without recruiting additional patients or incurring additional genotyping costs, we were able to include proctitis as a third form or radiation-induced injury.

During the first year of the project we ran a pilot set of 5 Affymetrix 6.0 microarrays to confirm the quality of our DNA samples and check the protocol for the arrays. We achieved over 99% call rates with these 5 pilot samples. We had previously run 83 Affymetrix 6.0 arrays on a separate patient set and were able to use the quality control results from this set to make adjustments to our protocol, resulting in the high DNA quality and genotyping call rates for the pilot samples from the current study.

We established assays in our laboratory for the validation of the SNPs and CNPs that appeared significantly associated with either urinary morbidity or erectile dysfunction in the initial training set and have successfully SNP and CNP genotyped patient samples. Through this work, we discovered that the SNPlex assay was not optimal for the genotyping to be performed and determined that more robust results were obtained using the TaqMan assay which also has an important advantage in that over 4.5 million assays are available. Since we have been successful with the use of TaqMan for SNP genotyping, we also decided to use TaqMan copy number assays for CNP analysis. TaqMan copy number assays consist of a TaqMan minor groove binding probe labeled with FAM dye and unlabeled PCR primers. The assays are run simultaneously with a copy number reference assay. The copy number assay detects the target genomic sequence of interest while the reference assay detects a sequence that is known to be present in two copies in the diploid genome. Relative quantitation analysis is performed using a known calibrator sample. As noted below, subsequently we chose a higher throughput method of SNP and CNP analysis.

Year 2: Efforts in the second year of funding were focused on completion of the discovery phase of the genome-wide association study. Genomic DNA from the 386 prostate cancer patients identified as cases or controls for one or more of the three outcomes of the study was assayed using Affymetrix SNP6.0 arrays.

A considerable amount of effort was spent on quality control checks to ensure sample identity and to assess and minimize risks of batch effects and population stratification. The 386 samples were run in 5 batches (i.e. 5 96-well plates). We incorporated two types of controls for each batch: an external control set comprising a HapMap trio (two parents and an offspring) and an internal control set comprising three duplicates of randomly selected prostate cancer patient samples. Initial overall genotyping rate among all 411 samples (study samples plus controls) was > 97%. We were able to confirm >99% reproducibility of genotype calls among the four batches by comparing the HapMap samples across batches. We also calculated identity-by-descent (IBD) and identity-by-state (IBS) measures to confirm the identity of the control samples and identify any patient samples with greater-than-expected similarity. We obtained expected IBD and IBS values for all controls: approximately 50% IBD sharing between the offspring and each parent of the HapMap trios, and >98% IBS sharing between identical pairs for all duplicate samples. Several prostate cancer samples were excluded based on greater-than-expected IBD sharing (8 pairs of samples) or low call rate (<90%; 2 samples). The final dataset contained 365 samples with call rate >98%.

Because the study involved a multi-ethnic patient population, the genetic population structure was assessed using principle components analysis and ancestry estimation using the program STRUCUTRE v2.1. As expected, based on self-reported race/ethnicity, approximately 78% of patients share ancestry primarily with Caucasian populations, approximately 4% share ancestry with Asian (Chinese and Japanese) populations, and approximately 18% are admixed with ancestry shared between African and Caucasian populations. For several patients with missing data on race/ethnicity, estimation of ancestry using SNP genotypes allowed us to accurately assign proportion shared ancestry and include those individuals in the analysis. We found no statistically significant differences in ancestry between cases and controls for any of the three outcomes suggesting that despite using a multi-ethnic cohort, we were able to adequately match cases and controls on race/ethnicity, thereby minimizing confounding by this variable in our association tests. To further minimize potential confounding by race/ethnicity, we used the estimated proportion ancestry as a variable in regression models to check that any significant associations identified were not strictly due to population stratification.

Association tests were carried out for each outcome using logistic regression models for ED and proctitis and linear regression models for urinary morbidity, adjusting for ancestry using the first five principle components. Analysis included 135 ED cases and 121 controls, 76 proctitis cases and 291 controls, and 347 patients with urinary symptom score measurements. We investigated four possible genetic inheritance models: allelic, genotypic, dominant and recessive. As outlined in our proposal, we set a fairly liberal cut-point of $p < 10^{-4}$ for inclusion in the validation study. This two-stage study design allowed us to capture most true positive associations and then filter out false positive associations through the validation study. Using the lowest p-value between the different inheritance models for each SNP, we identified 157 SNPs associated with urinary morbidity (p-values $6x10^{-7}$ to 10^{-4}), 167 SNPs associated with ED (p-values $2x10^{-7}$ to 10^{-4}), and 365 SNPs associated with proctitis (p-values $6x10^{-14}$ to 10^{-4}) that were investigated further in the validation study. As described below, improvements in technology allowed us to include a larger number of SNPs that these selected initially for investigation in the validation cohort.

Year 3: Efforts in the third year of funding were focused on the validation phase of the genome-wide association study. From the Discovery phase, completed during the second year of funding, we identified approximately 5,000 SNPs and CNP markers associated with one or more radiation-induced adverse effects under investigation. We then designed a custom SNP array that was created by Illumina (San Diego, CA), and we recently completing genotyping among 493 patients comprising the validation cohort.

Advances in technology that took place during the second year of the project allowed us to increase both our SNP selection limit and sample size for the validation study. For similar cost to doing TaqMan assays as planned, we were able to build a custom microarray using Illumina's Infinium iSelect HD custom genotyping platform to genotype samples in the validation cohort. This allowed us to select approximately 1% of the

SNPs from the discovery cohort for validation rather than the more modest numbers that would have been feasible using the TaqMan assay. Furthermore, for the same cost, we were also able to increase our sample size for the validation cohort from ~300 to 595 patients. Table 2 describes the patients selected for inclusion in the validation study.

Because the custom array allowed us to select a higher proportion of SNPs, we were able relax our otherwise conservative SNP selection criteria. Initially, we had set the type I error rate at 0.0001, allowing us to detect SNPs and CNPs with effect size of ~2.5 or greater. This would have resulted in selection of approximately 0.1% of the SNPs for genotyping in the validation study. However, since the custom microarray allowed us to type approximately 1% of the SNPs investigated in the discovery study, we were able to lower our type I error rate to 0.001, thereby allowing us to include SNPs that would have been otherwise thrown out as false negatives using the more stringent type I error threshold. We recognized that we were also increasing the number of false positives that would be carried over into the validation study, but we were confident of our ability to distinguish the true positives using a joint analytic approach whereby the p-values from the discovery and validation phases are combined to increase power [Skol 2006].

Table 2.

	Discovery Cohort N = 367	***Validation Cohort N = 493***
Age (yrs), mean(sd)	64 (7.3)	66 (7.5)
Stage, n(%)		
T1	200 (54.5%)	239 (48.7%)
T2	154 (42.0%)	233 (47.5%)
T3	13 (3.5%)	19 (3.9%)
Gleason, n (%)		
≤ 6	293 (65.1%)	279 (56.7%)
7	96 (26.2%)	141 (28.7%)
≥ 8	32 (8.7%)	72 (14.6%)
Pre-RT PSA (ng/ml), mean(sd)	9.4 (17.6)	8.6 (7.8)
Prostate CT volume (mm_3), mean(sd)	46.1 (17.6)	47.3 (17.9)
Prostate D90 (Gy), mean(sd)	150.4 (46.5)	149.0 (44.5)
Total BED (Gy), mean(sd)	203.3 (22.5)	199.6 (30.2)
RT type, n(%)		
Brachytherapy	204 (55.6%)	246 (50.0%)
Brachytherapy + EBRT	163 (44.1%)	226 (45.9%)
EBRT	1 (0.3%)	20 (4.1%)
Hormone therapy, n(%)	194 (52.9%)	262 (53.4%)
Smoking status n(%)		
Yes	141 (38.4%)	220 (44.6%)
No	226 (61.6%)	273 (55.4%)
Diabetes, n(%)	19 (5.2%)	37 (7.5%)
Hypertension, n(%)	131 (35.7%)	164 (33.3%)
Follow-up (months), mean (sd)	47.9 (12.5)	44.2 (14.8)

In our proposal, in addition to selecting SNPs found to be significantly associated with radiation adverse effects in the discovery phase, we included SNPs that are likely to affect genes functionally involved in radiation response. To this end, we worked with collaborators from Washington University School of Medicine in St. Louis and collaborators from the University of Cambridge in the UK to select such candidate SNPs. Specifically, we included in the validation study 104 SNPs that lie in genes that have been shown in published studies to play a role in radiation response pathways such as DNA damage repair, inflammation, and apoptosis. We also included 95 SNPs that were identified recently in the discovery phase of a similar GWAS currently underway and shared with us by our collaborators at the University of Cambridge. Because this study involves a multi-ethnic patient population, and ancestry was adjusted for in the analysis of the discovery phase data, we have selected approximately 1,000 ancestry-informative markers for inclusion on the custom array being used in the validation study. To do this, we performed principle components analysis using reference populations from three sources: the International HapMap Project, the Population Reference Sample (POPRES), and the Human Genome Diversity Project (HGDP)[Consortium 2003, Nelson 2008, Cavalli-Sforza 2005]. We selected SNPs with minor allele frequency differences between pairs of reference populations, and then, using principle components analysis, tested the ability of various sized panels of selected 'ancestry-informative' SNPs to distinguish the ethnically and geographically distinct reference populations. We compared the performance of our ancestry-informative SNPs to a random selection of 100,000 SNPs which is typically used for principle components analysis. We found that we could adequately stratify population groups using approximately 950 SNPs. These SNPs were included on the custom array and will be used in the validation study to calculate principle components for ancestry-adjustment in regression models.

We began building the custom SNP arrays in June, and, using the services of the Institute for Genomics and Multiscale Biology at Mount Sinai School of Medicine, in January 2012 completed genotyping all 595 patients in the validation cohort. We were granted a no-cost extension of one year, and are spending this time on final analysis of the data from the validation study and manuscript preparation. In preparation for this, we have finalized our analyses of clinical predictors of radiation adverse effects that will be included in the SNP analysis. Patient-related variables include age, pre-treatment symptoms (urinary symptoms and erectile function), use of hormone therapy, hypertension, diabetes, and smoking status. Treatment-related variables include total biologically effective dose, prostate D90 (minimum dose to 90% of the prostate volume), and whether the patient received external beam RT in addition to brachytherapy. We are currently in the process of completing QC checks on the validation cohort data, and will then begin statistical analysis using multivariate regression models to investigate each SNP as well as combinations of significant SNPs.

KEY RESEARCH ACCOMPLISHMENTS:

Year 1:

Refined and finalized inclusion criteria and case definitions for patients to be included in the study

Verified IPSS and SHIM/MSEF scores for a minimum of one year for all patients included in the study

Analyzed demographic and clinical characteristics of patients for whom we have blood collected to ensure similarity of cases and controls for each outcome

Established assays in our laboratory for the validation of the SNPs and CNPs that appear significantly associated with either urinary morbidity or erectile dysfunction in the initial training set and have successfully SNP and CNP genotyped patient samples.

Year 2:

Ran SNP/CNP genotyping arrays for 411 patient samples and controls in the discovery cohort

Achieved >98% call rate in final set of 367 patients after performing QC steps

Confirmed cases and controls were matched on race/ethnicity for all three outcomes and obtained ancestry estimates for each patient to include in logistic regression models for SNP association

Identified approximately 700 SNPs associated with ED, urinary morbidity or proctitis to be investigated in the validation cohort

Completed patient recruitment to fulfill the sample size requirement for the validation study

Year 3:

Designed and built a mid-plex custom SNP microarray to genotype approximately 5,000 SNPs identified in the discovery phase of the project as well as approximately 1,000 ancestry-informative markers

Worked with collaborators in the UK and US to select approximately 200 additional candidate SNPs on the basis of functional involvement in radiation response

Genotyped approximately 600 patients comprising the validation cohort for discovery phase SNPs and candidate SNPs

Developed regression models incorporating clinical and covariate that will be used to analyze each SNP in the validation study

Submitted the results of the study in abstracts at the 54th annual meeting of the American Society of Therapeutic Radiation Oncology (ASTRO)

REPORTABLE OUTCOMES

We have identified 157 SNPs associated with urinary morbidity (p-values $6x10^{-7}$ to 10^{-4}), 167 SNPs associated with ED (p-values $2x10^{-7}$ to 10^{-4}), and 365 SNPs associated with proctitis (p-values $6x10^{-14}$ to 10^{-4}) that are being investigated further in the validation study.

CONCLUSIONS

We have performed a genome-wide association study to identify genetic variants associated with radiotherapy adverse response. The results of this study will provide the basis for development of a clinically relevant predictive test to identify patients at increased risk for development of adverse events following radiotherapy. Such a tool could be used to aid clinicians in personalizing dosage to improve the therapeutic index of radiotherapy treatment for prostate cancer.

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APPENDICES

Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B. *Genome Wide Association Study to Identify Genetic Variants Associated with Urinary Symptoms Following Radiotherapy for Prostate Cancer.* Abstract submitted to the 54th annual ASTRO meeting (2012).

Buckstein M, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B. *Genome Wide Association Study to Identify Genetic Variants Associated with the Development of Erectile Dysfunction Following Radiotherapy for Prostate Cancer.* Abstract submitted to the 54th annual ASTRO meeting (2012).

Ko E, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B. *Association of Genetic Factors with PSA Response in Prostate Cancer Patients Receiving Definitive Radiotherapy.* Abstract submitted to the 54th annual ASTRO meeting (2012).

Genome Wide Association Study to Identify Genetic Variants Associated with Urinary Symptoms Following Radiotherapy for Prostate Cancer

Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B

Purpose/Objectives: Brachytherapy and external beam radiation achieve high cure rates for prostate adenocarcinoma. Though treatment delivery has improved over time, many patients still experience some form of late urinary symptoms that significantly impact quality of life. Even after controlling for clinical factors, considerable variability in toxicity is observed suggesting a genetic component. A predictive tool including genetic factors would assist in personalizing treatment. We performed a two-stage genome wide association study (GWAS) to identify genetic factors associated with urinary morbidity following radiotherapy for prostate cancer.

Methods: Prostate cancer patients treated with brachytherapy alone or brachytherapy plus external beam radiation therapy (EBRT) were assessed for urinary morbidity as measured by change in International Prostate Symptom Score (IPSS) from baseline. A total of 783 patients who had baseline IPSS available and > 1 year of follow-up were included. The change in IPSS was assessed at each 6-month follow-up interval between 1 year and 5 years post-treatment and evaluated as a quantitative trait in genetic association tests. Genotyping was done in two stages with patients split randomly into a discovery cohort (N=347) and a replication cohort (N=436). The discovery cohort was genotyped for ~900,000 SNPs using Affymetrix v6.0 arrays. The 1,480 SNPs most strongly associated with urinary morbidity were then selected for genotyping in the replication cohort using an Illumina custom array. Multivariate linear regression was used to test for association between each SNP and change in IPSS while controlling for pre-treatment IPSS, hypertension and race/ethnicity. Four different genetic inheritance models were investigated for each SNP: allelic, genotypic, dominant and recessive. Combined p-values were calculated for the discovery and replication studies using Fisher's method after filtering on agreement in effect direction.

Results: Several genomic regions were identified that contained clusters of SNPs with combined p-values reaching significance (1E-05 after correction for multiple comparisons). Interestingly, some of the significant SNPs were more strongly associated with early onset of urinary morbidity (between 1 year and 3 years post-treatment), whereas other significant SNPs showed a stronger association with later onset of urinary morbidity (between 3 years and 5 years post-treatment).

Conclusions: This study identifies several potential predictive genetic variants that are associated with urinary morbidity following prostate radiotherapy and could potentially be used to predict the severity of urinary symptoms for individuals receiving radiotherapy for prostate cancer.

Genome Wide Association Study to Identify Genetic Variants Associated with the Development of Erectile Dysfunction Following Radiotherapy for Prostate Cancer

Buckstein M, Kerns S, Stock R, Stone N, Ostrer H, Rosenstein B

Purpose/Objectives: Brachytherapy and external beam radiation therapy (EBRT) afford high rates of local control for prostate adenocarcinoma but carry the risk of late toxicities including erectile dysfunction (ED). When controlling for treatment characteristics, considerable variability in toxicity is observed suggesting a genetic component. A predictive tool including genetic factors would assist in weighing the benefits of radiation with the risks of chronic side effects. We performed a two-stage genome wide association study (GWAS) to identify genetic factors predictive for developing ED.

Methods: Prostate cancer patients treated with brachytherapy alone or brachytherapy plus EBRT were genotyped and selected for development of ED. ED was evaluated using the Sexual Health Inventory for Men (SHIM) questionnaire administered before treatment and during follow-up every 6 months. Patients were required to be potent prior to treatment (SHIM ≥ 16) and have ≥ 1 year follow up. Androgen Deprivation Therapy was allowed, but patients with persistent castrate-level testosterone were excluded. ED cases were defined by any post-treatment SHIM ≤ 7, and controls were defined by post-treatment SHIM ≥ 16 for all follow-up visits up to 5 years post-treatment. Genotyping was done in two stages with patients split randomly into a discovery cohort (132 cases and 103 controls) and a replication cohort (128 cases and 102 controls). From the results of the discovery GWAS in which ~900,000 SNPs were genotyped using an Affymetrix v6.0 array, 930 SNPs most strongly associated with ED were selected for follow-up genotyping in the replication cohort using an Illumina prostate custom array. Multivariate logistic regression was used to test for association between each SNP and ED while controlling for age, hormone use, EBRT, and race/ethnicity. Four different genetic inheritance models were investigated for each SNP: allelic, genotypic, dominant and recessive. Combined p-values were calculated for the discovery and replication studies using Fisher's method after filtering on agreement in effect direction.

Results: We identified 5 genes possessing a total of 8 SNPs that each exhibited a combined p-value, using the discovery and replication cohorts, less than 10^{-4} for association with ED. The combined odds ratios for these SNPs range from 1.99 (95% CI 1.45 – 2.74) for the SNP with the smallest effect to 3.16 (95% CI 1.89 – 5.29) for the SNP with the largest effect.

Conclusions: This study identifies several potential predictive genetic variants that are associated with ED following prostate radiotherapy. This work was supported by grants PC074201 from the DOD Prostate Cancer Research Program and 1R01CA134444 from NIH.

Association of Genetic Factors with PSA Response in Prostate Cancer Patients Receiving Definitive Radiotherapy

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Purpose/Objectives: Following definitive radiotherapy for prostate cancer, patients that attain a rapid response to a PSA nadir have been shown to have significantly better long-term clinical outcomes. Aside from treatment parameters, genetic factors are hypothesized to influence post-treatment PSA outcomes. We utilized a two-stage genome-wide association study (GWAS) to identify single nucleotide polymorphisms (SNPs) associated with time to PSA nadir.

Methods: We identified a cohort of 345 patients with low and intermediate risk prostate cancer who received brachytherapy with or without external beam radiation therapy between 1994-2008. None of these patients received androgen-deprivation therapy. In our two-stage analysis, patients who achieved PSA nadir (defined as PSA<0.1, <0.2, <0.3, or <0.5ng/ml) were randomly assigned to the discovery (n=170) or validation (n=175) cohorts, with equal weighting of pretreatment and treatment variables known to be associated with PSA outcomes. In the discovery phase, 900,000 SNPs were genotyped using an Affymetrix v6.0 array, and multivariate linear regression was used to test for associations between these SNPs and time to PSA nadir, while controlling for race/ethnicity. In the validation phase, a parallel multivariate linear regression was performed with a subset of 398 SNPs genotyped with an Illumina prostate custom array. Four different genetic inheritance models were tested for each SNP: allelic, genotypic, dominant and recessive. Combined p-values were calculated using Fisher's method.

Results: Median follow up for all patients was 75mos (range 10-215mos). 95%, 90%, 85%, and 72% of patients in the discovery cohort and 94%, 90%, 82%, and 73% of patients in the validation cohort attained a PSA nadir of <0.5, <0.3, <0.2, and <0.1ng/ml, respectively. Median post-treatment intervals to attain these PSA nadirs were comparable between cohorts and were 20mos(range 0.6-116mos), 28mos (range 0.6-116mos), 34mos (range 0.6-116mos), and 41mos (range 3-118mos), respectively. In combined analysis of the discovery and validation cohorts, we identified several SNPs that were significantly associated with a rapid interval to PSA nadir (combined p-values 10^-7 to 10^-4). In multivariate analysis with pretreatment (initial PSA, clinical stage, Gleason score) and treatment (BED) covariates, the identified SNPs were independently predictive of interval to PSA nadir.

Conclusions: We identified a panel of candidate SNPs that were strongly associated with time to PSA nadir following definitive prostate radiotherapy. Since the time to PSA nadir has been shown to be significantly associated with long-term clinical outcomes (e.g., freedom from biochemical failure and distant metastasis), our results suggest that at least some of these SNPs may be prognostically useful in the setting of prostate radiotherapy.